OIPE
MAR 0 9 2009
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Shizuka UEHARA et al.

Group Art Unit: 1655

Appln. No. : 10/531,289
(National Stage of PCT/JP2003/09651)

Examiner: Hoffman

I.A. Filed : July 30, 2003

Confirmation No: 4829

For : EXTERNAL PREPARATION FOR SKIN AND METHOD OF USING THE SAME

DECLARATION UNDER 37 C.F.R. 1.132 OF CHIHARU KOIDE

Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window, Mail Stop Amendment
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir:

I, the undersigned, Chiharu Koide, a citizen of Japan, do solemnly declare as follows:

1. That my academic career includes a B.M. from the Faculty of Pharmaceutical Sciences, Chiba University from 1976 to 1981, and an M.S. from the Graduate School of Pharmaceutical Sciences, Chiba University from 1981 to 1983. I have been employed by KOSE Corporation since 1983, with my positions including Researcher, material Research Section, Advanced Cosmetic Research Laboratories, Research Laboratories; Manager, Regulatory Affairs Section, Product Management Department, Research Laboratories; and Principal Researcher, Material Research Section, Advanced Cosmetic Research Laboratories, Research Laboratories.

2. That I have reviewed the Final Office Action mailed October 9, 2008 as well as the application that was filed in connection with the above-identified application when entering the national stage in the U.S. on April 14, 2005, published as WO 2004/041236 A1 (and hereinafter referred to as "the originally filed application".).

3. That experiments have been conducted, with my consultation, and these experiments have been described in the originally filed application in Examples 1, 2, 3, 5, 22, 29 and 32, and illustrated in Tables 1-1, 2-1, 3-1 and 3-2.

4. That these experiments include testing of Melanin Generation (Formation) Suppression and Cell Survival Rate Test based on Cell Culture; and Cell Activation Test based on Cell Culture, and are described in the following paragraphs and in the originally filed application.

5. That the following Melanin Generation (Formation) Suppression and Cell Survival Rate Test based on Cell Culture was conducted in a manner according to Example 3 as disclosed in the originally filed application, as follows:

Murine cultured B16 melanoma cells were used. An appropriate quantity of a 10% FBS-containing MEM medium was placed in two 6-well plates, the B16 melanoma cells were seeded therein and allowed to stand at 37°C under a carbon dioxide concentration of 5 vol%. Next day, a sample preparation solution was added and mixed therewith so as to adjust the final concentrations of the *Cistus ladaniferus* L. extract obtained in Example 1 as disclosed in the originally filed application (as described below) and, and labdenic acids and methyl esters and ethyl esters thereof obtained in Example 2 as disclosed in the originally filed application (as described below) to 0 (reference), 5 and 10 μg/mL, and of lily extract (product of Maruzen Pharmaceuticals Co., Ltd.), which is a known whitening agent, to 0 and 100 μg/mL. The

medium was exchanged on the fifth day of culture, and the sample preparation solution was added again. The medium was removed next day, the cells were collected from one plate after washing them using a phosphate buffer (pH7), and degree of whitening of the cultured B16 melanoma cells was evaluated according to the criteria shown below.

Similar test was conducted, as a comparative example, also using *Coix lachryma-jobi* extract (100 μg/mL), already known to have a suppressive effect over melanin formation.

Coixlachryma-jobi extract was obtained by adding 100 mL of a 70 vol% water-containing ethanol to 10 g of *Coixlachryma-jobi* (Japan Pharmacopoeia), carrying out extraction at room temperature for 3 days, and by filtering the mixture. Dry solid content of the *Coixlachryma-jobi* extract was found to be 0.8%.

(Criteria for Judgment)

++: distinctively stronger whiteness over the reference;

+: apparently stronger whiteness over the reference;

±: slightly stronger whiteness over the reference; and

-: remained unchanged.

On the other plate, the cells were fixed with formalin, and dyed by adding an 1% crystal violet solution. Cell survival rates for the individual sample concentrations were measured using a monocellator (product of Olympus Corporation). Results are shown in Modified Table 1-1 below which includes the same data as Table 1-1 in the originally filed application, but reformatted with the data rearranged to present the data in a clearer manner and with A denoting the *Cistus ladaniferus* L. extract, and B2 denoting the lily extract.

Preparation of *Cistus ladaniferus* L. Extract

Twenty kilograms of crushed leaves and twigs of *Cistus ladaniferus L.*, a plant of *Cistaceae*, were deoiled by steam distillation. The mixture was extracted with 200 kg of *n*-hexane, the obtained extract was distilled under reduced pressure so as to remove low-boiling-point components, to thereby obtain 150 g of extract in a solid to paste form.

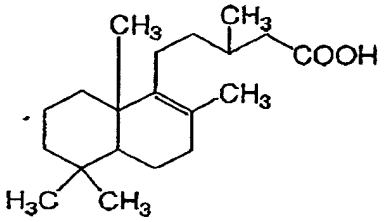
Preparation of labdenic acids, and their methyl esters and ethyl esters

Ten grams of commercial Labdanum Absolute (product of Givaudan) were subjected to molecular distillation under reduced pressure (0.1 mmHg), and a fraction (4.3 g) was collected over a range from 180°C to 220°C. The fraction was found to contain compound 1 (labd-8-en-15-oic acid), compound 4 (labd-7-en-15-oic acid) and compound 7 (labd-8(17)-en-15-oic acid) (the mixture is referred to as "acid mixture", hereinafter), shown below. One gram of the acid mixture was dissolved into 2 ml of ether, an ether solution of diazomethane is dropped therein, and thereby 0.96 g of a methyl ester product was obtained (the methyl ester product is referred to as "methyl ester mixture", hereinafter). Similarly, 10 g of the acid mixture was dissolved into 100 mL of ethanol, allowed to proceed esterification under the presence of a sulfuric acid catalyst, to thereby obtain 9.5 g of ethyl ester product (the ethyl ester product is referred to as "ethyl ester mixture", hereinafter).

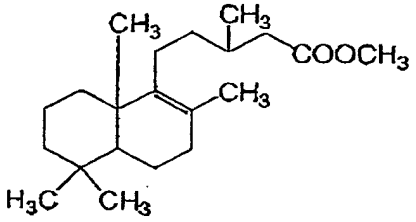
Next, the ethyl ester mixture was chromatographed on silica gel to thereby separate it into three acids. More specifically, 10 g of the ethyl ester mixture was dissolved into 100 mL of hexane, injected to a silver-nitrate-treated silica gel column, which was followed by injection of solvents and elution. The solvents injected were hexane for the beginning, and hexane added with 1 vol% of ethyl acetate for the next. Compound 3 (labd-8-en-15-oic acid ethyl ester) was

eluted first, and compound 6 (labd-7-en-15-oic acid ethyl ester) and compound 9 (labd-8(17)-en-15-oic acid ethyl ester) followed in this order. The solvent were removed from each of the eluates, to thereby obtain pure products of the individual ethyl esters (0.83 g, 0.16 g and 0.63 g in this order of elution). Thus-obtained ethyl ester products were hydrolyzed according to a general method, to thereby obtain free acids. The free acids were further added by dropping with an ether solution of diazomethane, and the solvent was distilled off, to thereby obtain the methyl ester products.

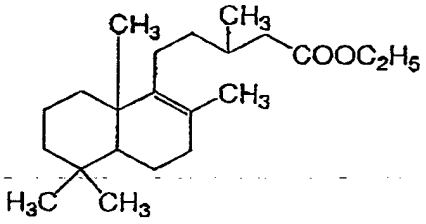
Compound 1



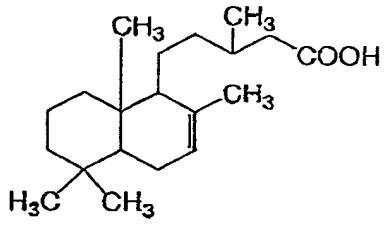
Compound 2



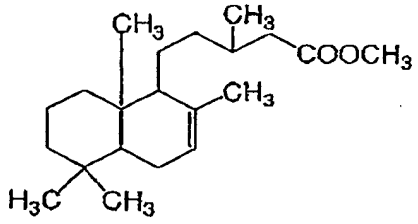
Compound 3



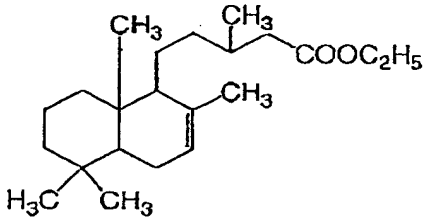
Compound 4



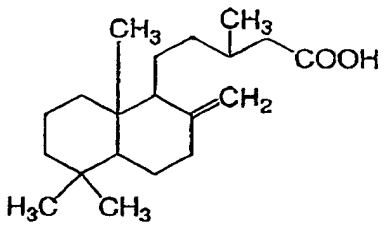
Compound 5



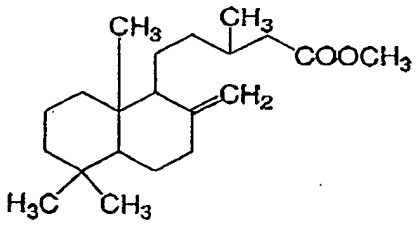
Compound 6



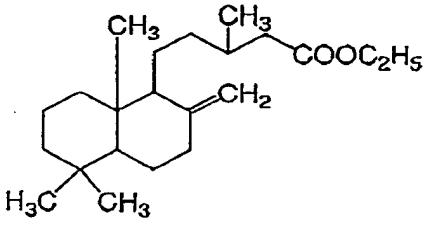
Compound 7



Compound 8

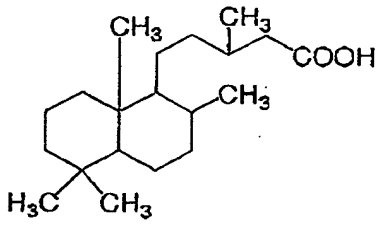


Compound 9

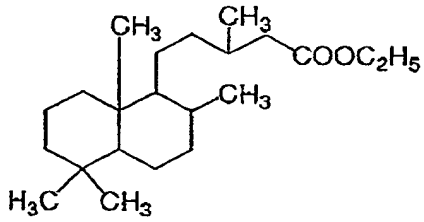


Then 4.3 g each of the ethyl ester products obtained in Example 2 was dissolved into 10 mL of ethanol, added with 0.2 g of a 5%-palladium carbon catalyst so as to proceed hydrogen addition reaction, to thereby obtain 4.1 g of compound 11. The product was further hydrolyzed to obtain compound 10.

Compound 10



Compound 11



6. That the following Melanin Formation (Generation) Suppression and Cell Survival Rate Test based on Cell Culture was conducted in a manner according to Example 22 as disclosed in the originally filed application, as follows:

Murine cultured B16 melanoma cells were used. An appropriate quantity of a 10% FBS-containing MEM medium was placed in two 6-well plates, the B16 melanoma cells were seeded therein and allowed to stand at 37°C under a carbon dioxide concentration of 5 vol%. Next day, a sample preparation solution was added and mixed therewith so as to adjust the final concentrations of the *Cistus ladaniferus* L. extract obtained in Example 1, and labdenic acids and methyl esters and ethyl esters thereof obtained in Example 2 to 0 (reference), 5 and 10 μg/mL, and of *Glycyrrhiza glabra* extract (product of Maruzen Pharmaceuticals Co., Ltd.), which is a known whitening agent, to 0 and 100 μg/mL. The medium was exchanged on the fifth day of culture, and the sample preparation solution was added again. The medium was removed next day, the cells were collected from one plate after washing them using a phosphate buffer (pH7),

and degree of whitening of the cultured B16 melanoma cells was evaluated according to the criteria shown below.

Similar test was conducted, as a comparative example, also using *Coix lachryma-jobi* extract (100 μg/mL), already known to have a suppressive effect over melanin formation.

Coixlachryma-jobi extract was obtained by adding 100 mL of a 70 vol% water-containing ethanol to 10 g of *Coixlachryma-jobi* (Japan Pharmacopoeia), carrying out extraction at room temperature for 3 days, and by filtering the mixture. Dry solid content of the *Coixlachryma-jobi* extract was found to be 0.8%.

Criteria for Judgment

++: distinctively stronger whiteness over the reference;

+: apparently stronger whiteness over the reference;

±: slightly stronger whiteness over the reference; and

-: remained unchanged.

On the other plate, the cells were fixed with formalin, and dyed by adding an 1% crystal violet solution. Cell survival rates for the individual sample concentrations were measured using a monocellator (product of Olympus Corporation). Results are shown in Modified Table 1-2 below which includes the same data as Table 1-2 in the originally filed application, but reformatted with the data rearranged to present the data in a clearer manner with A denoting the *Cistus ladaniferus* L. extract, and B1 denoting the *Glycyrrhiza glabra* extract.

7. That the following Cell Activation Test based on Cell Culture was conducted in a manner according to Example 32 as disclosed in the originally filed application, as follows:

Human neonatal fibroblast NB1RGB was used. An appropriate quantity of medium was placed in a 24-well plate, the fibroblast NB1RGB was seeded therein and allowed to stand at 37°C under a carbon dioxide concentration of 5 vol%. Next day, a sample preparation solution was added and mixed therewith so as to adjust the final concentrations of the *Cistus ladaniferus* L. extract obtained in Example 1, and labdenic acids and methyl esters and ethyl esters thereof obtained in Example 2 to 0 (reference), 5 and 10 μg/mL, and of cactus extract (prepared according to Example 29 in the originally filed application and as described below), which is a known cell activator, to 0 and 100 μg/mL. The medium was exchanged on the fourth day of culture, and the sample preparation solution was added again. The medium was removed next day, the cells were washed using a phosphate buffer and collected, and evaluated in terms of cell proliferation rate based on comparison of the number of fibroblast NB1RGB cells grown in the individual sample preparation solutions with that of the reference.

Similar test was conducted, as a comparative example, also using soybean extract (100 μg/mL), already known to have a cell activation effect. Soybean extract was obtained by adding 100 mL of a 70 vol% water-containing ethanol to 10 g of soybean seed, carrying out extraction at room temperature for 3 days, and by filtering the mixture. Dry solid content of the soybean extract was found to be 0.5%.

Method of Preparing Cactus Extract according to Example 29 in the originally filed application.

Ten grams of dried and pulverized stem of *Opuntia Streptacantha* were added with 100 mL of purified water, and extracted at 70°C for 8 hours. The mixture was cooled and filtered, the filtrate was concentrated to dryness, added with 100 g of a 30 vol% water-containing 1,3-butylene glycol for solubilization, to thereby obtain a cactus extract (dry solid content: 1.0%).

Criteria for Evaluation

The number of cells grown in each sample preparation solution was compared with the number of cells of the reference, and cell activation effect was evaluated using cell proliferation ratio as an index. The number of cells was counted using a blood cell counter plate. Results are shown in Modified Table 3-2 below which includes the same data as Table 3-2 in the originally filed application, but reformatted with the data rearranged to present the data in a clearer manner with A denoting the *Cistus ladaniferus* L. extract, and B3 denoting the cactus extract.

8. That the following Cell Activation Test based on Cell Culture was conducted in a manner according to Example 5 as disclosed in the originally filed application, as follows:

Human neonatal fibroblast NB1RGB was used. An appropriate quantity of medium was placed in a 24-well plate, the fibroblast NB1RGB was seeded therein and allowed to stand at 37°C under a carbon dioxide concentration of 5 vol%. Next day, a sample preparation solution was added and mixed therewith so as to adjust the final concentrations of the *Cistus ladaniferus* L. extract obtained in Example 1, and labdenic acids and methyl esters and ethyl esters thereof obtained in Example 2 to 0 (reference), 5 and 10 μg/mL, and of *Panax ginseng* extract (product of Maruzen Pharmaceuticals Co., Ltd.), which is a known cell activator, to 0 and 100 μg/mL. The medium was exchanged on the fourth day of culture, and the sample preparation solution was added again. The medium was removed next day, the cells were washed using a phosphate

buffer and collected, and evaluated in terms of cell proliferation rate based on comparison of the number of fibroblast NB1RGB cells grown in the individual sample preparation solutions with that of the reference.

Similar test was conducted, as a comparative example, also using soybean extract (100 μg/mL), already known to have a cell activation effect. Soybean extract was obtained by adding 100 mL of a 70 vol% water-containing ethanol to 10 g of soybean seed, carrying out extraction at room temperature for 3 days, and by filtering the mixture. Dry solid content of the soybean extract was found to be 0.5%.

Criteria for Evaluation

The number of cells grown in each sample preparation solution was compared with the number of cells of the reference, and cell activation effect was evaluated using cell proliferation ratio as an index. The number of cells was counted using a blood cell counter plate. Results are shown in Modified Table 3-1 below which includes the same data as Table 3-1 in the originally filed application, but reformatted with the data rearranged to present the data in a clearer manner with A denoting the *Cistus ladaniferus* L. extract, and B4 denoting the Panax ginseng extract..

9. That synergistic effects in terms of whitening are shown in Tables 1-2 and 1-1.

That the data shown in Table 1-2 and 1-1 are results regarding "Melanin Production Suppression" and "Cell Survival Rate Test" based on cell culture. These tests are general tests in the technical field of external preparation for skin such as cosmetics to which the present invention is directed. According to the melanin-production-suppression test, whiteness of each sample is determined compared with a reference to which no ingredient has been added. When whiteness of each sample is determined, some indicators showing how whiteness should be judged as "++", "+", "±" or "-", are used, as illustrated in the photograph of indicators attached

as Exhibit 1. Therefore, the data comprises reproducible results using general tests in the technical field.

Furthermore, the degree of blackness of each sample is proportional to the concentration of melanin produced in cells, and so, more whiteness means a lower concentration of melanin. Therefore, data as disclosed in the originally filed application, and data as presented herein include quantifiable data.

In addition, actually, a whitening effect is judged visually by the user or the third person; and therefore, in terms of actual use, the results based on visual judgments are significant and sensible.

According to the test of "Melanin Formation (Generation) Suppression", generally, when an additional concentration of a whitening agent is increased, the degree of whiteness is improved. However, there is a limitation of the whitening ability of each agent, and therefore the degree of whiteness almost cannot be improved after reaching the certain degree even if the concentration is increased.

As can be seen from a review of the data shown in Tables 1-2 and 1-1, the following is applicable.

All of the samples, employing the combination of Ingredient (A) and Ingredient (B1) (Nos. B1-3a – 3g) or the combination of Ingredient (A) and Ingredient (B2) (Nos. B2-3a – 3g), show the highest degree of "++", on the other hand, all of the samples employing only Ingredient (A) (Nos. B1-2a – 2g and Nos. B2-2a – 2g) show the degree of "+".

The concentration of Ingredient (A) contained in each of the latter samples is double as compared with the amount of Ingredient (A) contained in each of the former samples (for

example, the concentration of (A) contained in sample B1-2a is 20 μg/mL, which is double compared with the concentration of (A) contained in sample B1-3a, 20 μg/mL).

Considering this point, it can therefore be said that the degree of "+" is the limit which can be achieved by Ingredient (A) alone; and that the degree "++", which cannot be achieved by Ingredient (A) alone, is achieved by the combination of Ingredient (A) and either of Ingredient (B1) or (B2).

All of the samples employing Ingredient (B1) or (B2) alone (Nos. B1-1a – 1g and Nos. B2-1a – 1g) showed the degree of "±".

The concentration of Ingredient (B1) or (B2) contained in each of the samples is double as compared with the amount of Ingredient (B1) or (B2) contained in each of the samples employing the combinations, Nos. B1-3a – 3g and Nos. B2-3a – 3g (for example, the concentration of (B1) contained in sample B1-1a is 200 μg/mL, which is double compared with the concentration of (B1) contained in sample B1-3a, 100 μg/mL).

Considering this point, it can be said that the degree "++", which cannot be achieved by Ingredient (B1) or (B2) alone, is achieved by the combination of Ingredient (A) and either of Ingredient (B1) or (B2) even if the concentration of (B1) or (B2) is smaller.

The synergistic whitening effect of the combination of Ingredient (A) and Ingredient (B1) or (B2) is therefore illustrated, and the such synergistic effect of the combinations would have been unexpected at the time the invention was made.

Generally, for obtaining the whitening effect in the actual use of a whitening agent, it is required not only the whitening ability but also the cell survival rate as shown in Table 1-2 and Table 1-1. In general, it is said that the higher concentration of a whitening agent results in the

lower cell survival rate; and therefore achieving a whitening effect with a smaller concentration of a whitening agent is more preferable.

Furthermore, Ingredient (A) is a hydrophobic compound and is hardly dissolved in an aqueous solvent. For preparing an external preparation for skin, it is necessary to dissolve various ingredients in an aqueous solvent. An external preparation for skin containing Ingredient (A), which is a poorly-soluble ingredient, may be required to show a high whitening effect with a small concentration of Ingredient (A). According to the invention, by employing the combination of Ingredient (A) and Ingredient (B1) or (B2), the excellent whitening effect, which cannot be achieved by each of ingredients alone, is achieved even with the smaller concentration of each of them. Considering this point, the present invention can also contribute to widening the range in choosing the formulation of external preparations for skin, and is very usable.

10. That the synergistic effects in terms of cell-activation (anti-aging) are shown in Tables 3-2 and 3-1.

The data shown in Tables 3-2 and 3-1 are results regarding "Cell Activation Test based on Cell Culture" which corresponds to an anti-aging effect. These tests are general tests in the technical field of external preparation for skin such as cosmetics that the present invention belongs to. According to the test, the cell-activation rate of each sample is calculated as follows.

$$\text{Cell activation rate} = \frac{\text{The number of cells in each sample containing ingredient(s)}}{\text{The number of cells in each sample not containing any ingredient}} \times 100$$

From the data shown in Tables 3-2 and 3-1, it is understood that the number of cells in each sample (Nos. B3-3a – 3g and Nos. B4-3a – 3g) employing the combination of Ingredient (A) and Ingredient (B3) or (B4) increased significantly compared with each sample employing Ingredient (A) alone or each sample employing Ingredient (B3) or (B4) alone. For example, the cell activation rates of the samples employing Ingredient (A) and Ingredient (B3) are 230, 290,

280, 305, 300, 310 and 315 %; and the cell activation rates of the samples employing Ingredient (A) and Ingredient (B4) are 250, 320, 310, 345, 329, 322 and 333%; on the other hand, the cell activation rates of the samples employing Ingredient (A) alone are 120, 130, 125, 135, 128, 130 and 135; and the cell activation rate of the sample employing Ingredient (B3) or (B4) is 110 %.

Therefore, it can be said that, by employing the combination of Ingredient (A) and Ingredient (B3) or (B4), the cell-activation rate, which corresponds to the anti-aging effect, became almost double or triple, compared with those achieved by the samples employing Ingredient (A), (B3) or (B4) alone. Therefore, the synergic anti-aging effect of the combination of Ingredient (A) and Ingredient (B3) or (B4) is explicit from the data described in the originally filed application, and the effect of the combinations would have been unexpected at the time the invention was made.

In addition, the combination of Ingredient (A) and Ingredient (B3) or (B4) can achieve the effect even if the concentration of Ingredient (A) or either of Ingredient (B3) or (B4) is decreased. Achieving an anti-aging effect with a smaller concentration of a anti-aging agent is preferable. By employing the combination of Ingredient (A) and Ingredient (B3) or (B4), the excellent anti-aging effect, which cannot be achieved by each of ingredients alone, is achieved even with the smaller concentration of each of them.

Modified Table 1-2 on pages 61-62 of the specification (showing the synergic effect of the combination (A) and (B1))

No.	Types of Ingredient (A)	Final concentration of Ingredient (A) [μg/mL]	Additional concentration of Ingredient (B1) Glycyrrhiza glabra extract [μg/mL]	Degree of whiteness	Cell survival rate (%)
B1-1a	Cistus ladaniferus L. Extract	0	20	±	98
B1-2a		20	0	+	100
B1-3a		10	10	++	102
B1-1b	Acid mixture	0	20	±	98
B1-2b		10	0	+	92
B1-3b		5	10	++	108
B1-1c	Methyl ester mixture	0	20	±	98
B1-2c		10	0	+	90
B1-3c		5	10	++	97
B1-1d	Ethyl ester mixtures	0	20	±	98
B1-2d		10	0	+	90
B1-3d		5	10	++	93
B1-1e	Compound 1	0	20	±	98
B1-2e		10	0	+	94
B1-3e		5	10	++	95
B1-1f	Compound 4	0	20	±	98
B1-2f		10	0	+	93
B1-3f		5	10	++	96
B1-1g	Compound 7	0	20	±	98
B1-2g		10	0	+	98
B1-3g		5	10	++	98
B1-1h	Coix lachrymal-jobi extract	0	20	±	98
B1-2h		200	0	±	100
B1-3h		100	10	++	97

Modified Table 1-1 on page 32 of the specification (showing the synergic effect of the combination (A) and (B2))

No.	Types of Ingredient (A)	Final concentration of Ingredient (A) [μg/mL]	Additional concentration of ingredient (B2) lily extract [μg/mL]	Degree of whiteness	Cell survival rate (%)
B2-1a	Cistus ladaniferus L. Extract	0	200	±	100
B2-2a		20	0	+	100
B2-3a		10	100	++	105
B2-1b	Acid mixture	0	200	±	100
B2-2b		10	0	+	92
B2-3b		5	100	++	120
B2-1c	Methyl ester mixture	0	200	±	100
B2-2c		10	0	+	90
B2-3c		5	100	++	98
B2-1d	Ethyl ester mixtures	0	200	±	100
B2-2d		10	0	+	90
B2-3d		5	100	++	89
B2-1e	Compound 1	0	200	±	100
B2-2e		10	0	+	94
B2-3e		5	100	++	92
B2-1f	Compound 4	0	200	±	100
B2-2f		10	0	+	93
B2-3f		5	100	++	94
B2-1g	Compound 7	0	200	±	100
B2-2g		10	0	+	98
B2-3g		5	100	++	95
B2-1h	Coix lachrymal-jobi extract	0	200	±	100
B2-2h		200	0	±	100
B2-3h		100	100	++	94

Modified Table 3-2 on page 68 of the specification (showing the synergic effect of the combination (A) and (B3))

No.	Types of Ingredient (A)	Final concentration of Ingredient (A) [μg/mL]	Additional concentration of Ingredient (B3) cactus extract [μg/mL]	Cell-activation rate (%)
B3-1a	Cistus ladaniferus L. Extract	0	200	110
B3-2a		20	0	120
B3-3a		10	100	230
B3-1b	Acid mixture	0	200	110
B3-2b		10	0	130
B3-3b		5	100	290
B3-1c	Methyl ester mixture	0	200	110
B3-2c		10	0	125
B3-3c		5	100	280
B3-1d	Ethyl ester mixtures	0	200	110
B3-2d		10	0	135
B3-3d		5	100	305
B3-1e	Compound 1	0	200	110
B3-2e		10	0	128
B3-3e		5	100	300
B3-1f	Compound 4	0	200	110
B3-2f		10	0	130
B3-3f		5	100	310
B3-1g	Compound 7	0	200	110
B3-2g		10	0	135
B3-3g		5	100	315
B3-1h	soybean extract	0	200	110
B3-2h		200	0	108
B3-3h		100	100	170

Modified Table 3-1 on page 39 of the specification (showing the synergic effect of the combination (A) and (B4))

No.	Types of Ingredient (A)	Final concentration of Ingredient (A) [μg/mL]	Additional concentration of Ingredient (B4) Panax ginseng extract [μg/mL]	Cell-activation rate (%)
B4-1a	Cistus ladaniferus L. Extract	0	200	110
B4-2a		20	0	120
B4-3a		10	100	250
B4-1b	Acid mixture	0	200	110
B4-2b		10	0	130
B4-3b		5	100	320
B4-1c	Methyl ester mixture	0	200	110
B4-2c		10	0	125
B4-3c		5	100	310
B4-1d	Ethyl ester mixtures	0	200	110
B4-2d		10	0	135
B4-3d		5	100	345
B4-1e	Compound 1	0	200	110
B4-2e		10	0	128
B4-3e		5	100	329
B4-1f	Compound 4	0	200	110
B4-2f		10	0	130
B4-3f		5	100	322
B4-1g	Compound 7	0	200	110
B4-2g		10	0	135
B4-3g		5	100	333
B4-1h	Soybean extract	0	200	110
B4-2h		200	0	108
B4-3h		100	100	170

The undersigned further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned application or any patent issuing thereon.

2009. 03. 03
Date

Chiharu Koide
Chiharu Koide

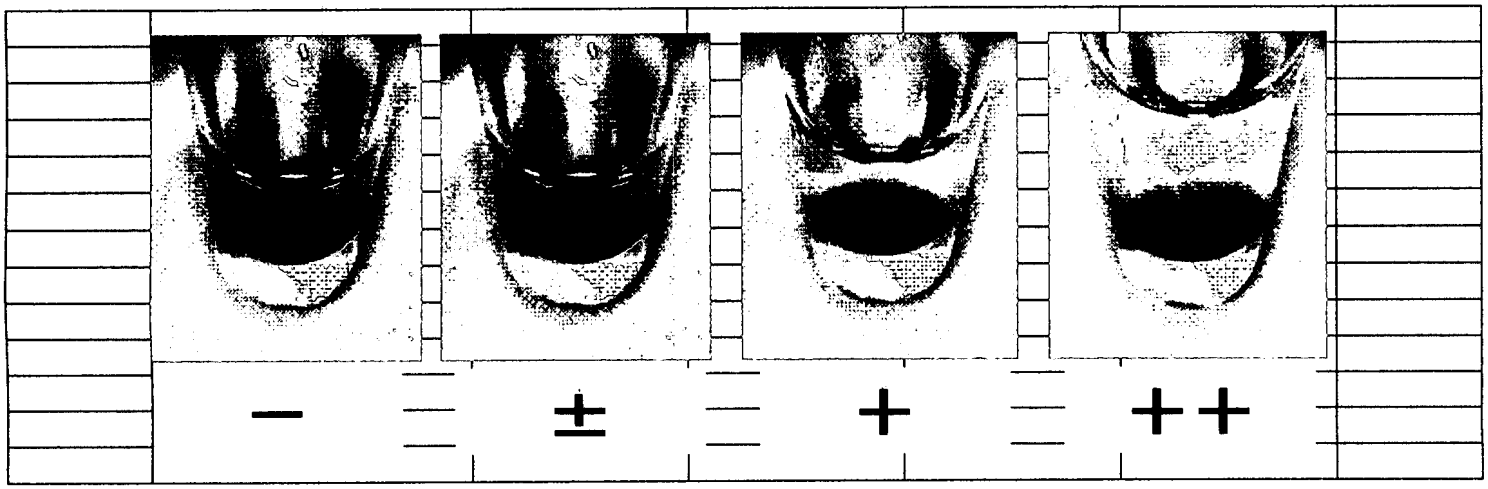


Exhibit 1